Challenges of Linking Early-Life Conditions and Disease Susceptibility

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Since David Barker first raised concerns about the impact of poor maternal nutrition and fetal growth restriction on the infant's long-term health (1,2), the obesity epidemic has shifted attention to the long-term effects of maternal over-nutrition and hyperglycemia in pregnancy (rev. in 3). Currently, one in five women is obese at the start of pregnancy and 2–10% are diagnosed with gestational diabetes mellitus (4). With the new diagnostic criteria for gestational diabetes mellitus, this number is expected to rise to ~18% of pregnant women worldwide (5,6). Despite these foreboding increases, we still have limited insight into the underlying mechanisms that link in utero conditions to later susceptibility for chronic disease. The inability to assess intrauterine exposures is one significant barrier to progress in this area. The discovery of a marker or profile that would more precisely measure fetal exposure to adverse conditions could ultimately help to identify infants at increased risk for age-related diseases and target preventative strategies specifically to at-risk individuals. In this edition of *Diabetes*, Bouchard et al. (7) tackle this problem in their investigation of placental changes in cytosine methylation associated with maternal glucose tolerance. At the same time, their work exemplifies some of the common challenges in epigenomic research.

Bouchard et al. report on a cohort of 98 French Canadian women who were assessed during pregnancy for markers of insulin resistance, glucose tolerance (using the oral glucose tolerance test), and adiponectin levels. At delivery, placental samples from the fetal side (intervillous and chorionic villi) and the maternal side (primarily fetal villous and deciduas basalis) were collected. Three CpG islands of the *ADIPOQ* locus were epigenotyped using pyrosequencing after bisulfite conversion. The investigators found increased 2-h glucose levels correlated with lower cytosine methylation levels in the *ADIPOQ* promoter on the fetal side of the placenta and increased maternal homeostatis model assessment of insulin resistance correlated with lower cytosine methylation levels in this promoter on the maternal side. The biologic plausibility of these findings is supported if one considers that maternal glucose crosses the placenta via facilitated diffusion, whereas maternal insulin does not cross to the fetus (7). The strengths of the study include the longitudinal study design and inclusion of a fairly large cohort of pregnant women who represent a continuum of glucose tolerance. Furthermore, continuous quantitative measurements, such as glucose levels, are not reduced to categorical variables (e.g., normal vs. impaired glucose tolerance), which could eliminate potentially relevant information.

Despite these strengths, the study is hampered by two significant limitations. First, the investigators rely on a single oral glucose tolerance test value at one point in a 40-week pregnancy as a marker of fetal exposure to hyperglycemia. The intrauterine environment is essentially a "black box" and as a result, surrogate markers of fetal exposures are commonly used. Most of these are crude in comparison with tools used for other measures, such as DNA sequencing or cytosine methylation. In this study, almost one-third of the participants had some intervention (i.e., diet or insulin therapy), which further obscures the degree of maternal glycemia that could affect the fetus. Other parameters, such as birth weight for gestational age or cord blood C-peptide levels, though also imperfect, may have provided better surrogate markers for cumulative glucose exposure. The second limitation of this study stems from interpretation of methylation changes in placental samples. The investigators use a reliable, well-validated technique for assessing site-specific DNA methylation. Quantitative differences in methylation (usually described as percent methylation) are meant to represent a shift in the methylation status at the specific locus of interest within a percentage of homogenous cells. Each cell type has a distinct methylation pattern and any given CpG site within a cell will be either methylated or unmethylated. Methylation changes found in mixed cell tissues, such as placenta may represent a simple shift in the proportion of cellular subpopulations without any actual change in methylation status. The methylation assay then becomes an inefficient tool as differences represent a differential cell count. Thus, some evaluation of the cellular composition becomes critical, particularly in placental samples that are apt to have variable patterns of angiogenesis and villous morphology (8).

Despite these limitations, the work by Bouchard et al. (7) is another step forward in the quest to understand the biological link between maternal health and long-term health of the unborn child. Its shortcomings exemplify the universal complexity of unraveling gene-environment interactions, about which three challenges have been defined. The first is a lack of high dimensional phenotyping (termed phenome [9]). Often, investigators examine a condition or disease defined by conventional unidimensional measures (e.g., maternal glucose levels) of characteristics that have already been judged to be important and fail to include other potentially relevant qualities, relationships, and measures in aggregate (rev. in 10). Secondly, we lack tools that enable accurate assessment of environmental exposures (11). These exposures are exceedingly difficult to define and nearly impossible to quantify, particularly in tissues or

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DOI: 10.2337/db12-0087

See accompanying original article, p. 1272.

organs that are inaccessible, such as the fetus or brain. Further, each exposure may be dynamic in nature, varying in time and space, and has the potential to influence health many years after the exposure(s) has occurred. The third missing component is a systems biology approach with an emphasis on predictive modeling. Feinberg (12) has described systems biology to include biological data sets (generally -omic based with various levels of hierarchy and iteratively defined interactions) that can be used to create comprehensive predictive models. In epigenomic research, focus is often placed on the integration of large data sets, which is itself a computational challenge. The next step is to create models of network interactions that could simulate behavior of the system based on a multitude of simultaneous, dynamic relationships as well as the probability of stochastic occurrences disrupting an expected cascade of events.

The advancement of our understanding of the developmental contributions to late-onset diseases will undoubtedly include continued technology development and expansion of computational and analytical capabilities. These challenges may seem insurmountable, much like the initial prospects of putting a man on the moon must have been. Perhaps progress will begin when we embrace the conceptual complexity of these challenges and resolve to conquer them.

ACKNOWLEDGMENTS

F.H.E. receives funding support from the National Institutes of Health (HD063791) and the Diabetes Research Center and the Nathan Shock Center of Excellence in the Biology of Aging at the Albert Einstein College of Medicine. The funders had no role in the decision to publish or the preparation of the manuscript.

No potential conflicts of interest relevant to this article were reported.

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